(12) UK Patent Application (19) GB (11) 2 185 317 (13) A

(43) Application published 15 Jul 1987

(21) Application No 8700696

(22) Date of filing 13 Jan 1987

(30) Priority data

(31) 8600681 (32) 13 Jan 1986 (33) GB

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(51) INT CL[4]
C12Q 1/00 1/32

(52) Domestic classification (Edition I)
G1B 710 740 AB
U1S 1116 1333 2135 2420 G1B

(56) Documents cited
None

(58) Field of search
G1B
Selected US specifications from IPC sub-class C12Q

(54) Improvements in or relating to the chemical analysis of a smoking related product

(57) The citrate content of cigarette paper containing citrate ions is determined on a discrete analyzer by extracting the citrate ions from the cigarette paper, enzymatically converting the extracted citrate ions to oxaloacetate and pyruvate ions, reducing the oxaloacetate and pyruvate ions by means of reduced nicotinamide-adenine dinucleotide (NADH) in the presence of a second enzyme catalyst, and measuring the amount of unoxidised NADH remaining, being proportional to the citrate content of the paper, by spectrophtotmetric techniques, at 340 nM.

The first enzyme is preferably citrate lyase and the second is preferably a mixture of malate dehydrogenase and lactate dehydrogenase.

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SPECIFICATION

Improvements in or relating to the chemical analysis of a smoking related product

This invention concerns improvements in or relating to the chemical analysis of a smoking-related product, in particular cigarette paper.

In the quality control of cigarettes or other smoking articles it has long been customary to carry out chemical analyses of tobacco and other smoking-related products such as filter rod material and cigarette paper. These analyses include analyses for naturally occurring sugars and nicotine in tobacco, glycerol triacetate (triacetin) in cellulose acetate based filter rod material, and citrate salts, which are used as burn modifiers, in cigarette paper.

Hitherto, such analyses have been carried out by a number of laboratory personnel, each assigned to a separate analytical task. It is now proposed to carry out simultaneous chemical analyses of tobacco or tobacco-related products for a number of constituents by means of a discrete analyzer (one example of which being the Technicon RA-1000), such as is used in the clinical analysis of physiological samples, but adapted to carry out chemical analyses specific to the tobacco industry. The use of a discrete analyzer enables analyses to be carried out simultaneously, more efficiently, more accurately, and more consistently, on one machine, than can be carried out by a number of separate analytical processes and operators.

There are restrictions imposed by the use of a discrete analyzer, namely that it is unable to support heating, filtration, or dialysis procedures, the use of corrosive materials, or analysis cycles involving lengthy time delays. Although the discrete analyzer is designed to avoid cross-contamination, in practice this is difficult to achieve completely, and a consequence of this is that the chemical constitution of the reagents used must be such that interaction is minimal. Furthermore, discrete analysis is not a closed system and is open to airborne contamination.

In a standard method for analysing cigarette paper for citrate content a citrate sample is extracted from the cigarette paper with water and reacted with acetic anhydride and pyridine to produce a red product. The spectral absorption of the red product is read at 385 nm (manual method) or 420 nm (using a Technicon Auto-Analyser Mark I). The citrate content of the sample is calculated by comparison with a calibration graph produced from standard citrate solutions.

Clearly, this standard analysis procedure is unacceptable for use on a discrete analyzer in conjunction with other analyses because of the risk of contamination of the other analyses by the acetic anhydride and pyridine used in the standard method.

According to the present invention there is provided a method of determining on a discrete analyzer the citrate content of cigarette paper containing citrate ions, the method comprising the steps of, extracting the citrate ions from the cigarette paper; in the discrete analyzer, by means of a first enzyme catalyst enzymatically converting the extracted citrate ions to a group of ions including oxaloacetate and pyruvate ions, in the presence of a second enzyme catalyst reducing the oxaloacetate and pyruvate ions by means of reduced nicotinamide-adenine dinucleotide (NADH), the amount of NADH so oxidised being proportional to the citrate content of the paper, measuring the concentration of unoxidised NADH; and deriving the citrate content of the paper from said concentration of unoxidised NADH.

The invention will now be described with reference to the following non-limiting example.

A citrate reagent A was made up containing the following constituents:
136U malate dehydrogenase (MDH)
248U lactate dehydrogenase (LDH)
12U lyophilized citrate lyase (CL)
6.0 mg reduced nicotinamide adenine dinucleotide (NADH)
5 drops wetting agent
and dissolved in 40 ml glycylglycine buffer to give a buffered enzyme mixture B of pH 7.8. 1U is the amount of enzyme required to catalyse 1 micromole per minute.

Citrate ions were extracted from cigarette paper by means of dilute hydrochloric acid to make an extract solution C.

12.5 microlitres of extract solution C were added to 350 microlitres of mixture B in a discrete analyzer and, after 6 minutes, the intensity of coloration was measured spectrophotometrically at 340 nm to find the amount of NADH present.

The same process was carried out on a citrate sample having a known concentration of citrate ions and the amount of NADH found was compared with that of extract C. The concentration of citrate in the cigarette paper was then calculated from the comparison.

In the invention CL converts citric acid or citrate to acetate and oxaloacetate ions. The oxaloacetate ion is further converted to pyruvate ion. In the presence of MDH and LDH, oxaloacetate and pyruvate ions are reduced by NADH to L-malate and L-lactate ions respectively. The decrease in the level of NADH due to its oxidation is directly proportional to the original citrate content in the cigarette paper. Hence NADH concentration monitored at 340 nm is inversely proportional to the citrate content of the sample.

A comparison of the method of the invention with the standard method described above (on a Technicon Mark I Auto-Analyser) was carried out on samples of different types of cigarette paper, the discrete analyzer analyses being repeated over a period of 5 weeks to test reproducibility of the analysis.

The results of Table 1 show percentage mixed citrate measurements of different types of cigarette paper as measured by the standard method and by the method of the invention carried out on the discrete analyzer, and

demonstrate that there was no significant difference between the results generated by the two methods and that consistent results were obtained by the discrete analyzer method of the present invention over a period of time.

TABLE 1

% Mixed citrate analysis

Paper Type	*Discrete Analyser*	*Auto-Analyser*				
		Week 1	*Week 2*	*Week 3*	*Week 4*	*Week 5*
1	0.05	0.06	0.06	0.09	0.10	0.07
2	0.00	0.04	0.05	0.09	0.11	0.08
3	0.28	0.30	0.30	0.34	0.33	0.31
4	0.29	0.29	0.32	0.33	0.34	0.33
5	0.52	0.53	0.54	0.56	0.56	0.55
6	0.68	0.67	0.70	0.70	0.71	0.68
7	0.99	1.04	1.05	1.01	1.01	1.00
8	1.98	2.02	2.03	1.96	1.95	1.93
9	2.03	2.07	2.15	2.02	2.06	2.01
10	1.96	2.07	2.09	1.97	1.97	1.94
11	2.06	2.09	2.18	2.06	2.07	2.05
12	1.93	2.05	2.04	1.93	1.95	1.91
13	1.98	2.04	2.10	2.01	2.02	2.00
14	3.14	2.80	2.91	2.76	2.79	2.74
15	3.20	3.12	3.16	3.03	3.03	2.97
16	3.30	3.36	3.50	3.30	3.32	3.27
17	4.00	4.61	4.64	4.37	4.38	4.34
18	1.27	1.19	1.22	1.16	1.15	1.15
19	1.18	1.20	1.27	1.20	1.20	1.19
20	1.13	1.26	1.30	1.23	1.23	1.22
21	1.55	1.54	1.68	1.61	1.59	1.61

CLAIMS

1. A method of determining on a discrete analyzer the citrate content of cigarette paper containing citrate ions, the method comprising the steps of, extracting the citrate ions from the cigarette paper; in the discrete analyzer, by means of a first enzyme catalyst enzymatically converting the extracted citrate ions to a group of ions including oxaloacetate and pyruvate ions, in the presence of a second enzyme catalyst reducing the oxaloacetate and pyruvate ions by means of reduced nicotinamide-adenine dinucleotide (NADH), the amount of NADH so oxidised being proportional to the citrate content of the paper, measuring the concentration of unoxidised NADH; and deriving the citrate content of the paper from said concentration of unoxidised NADH.

2. The method as claimed in claim 1 wherein the first enzyme catalyst is citrate lyase.

3. The method as claimed in claim 1 or claim 2 wherein the second enzyme catalyst is a mixture of malate dehydrogenase and lactate dehydrogenase.

4. The method as claimed in any one of claims 1–3 wherein the concentration of the unoxidised NADH is measured spectrophotometrically at a wavelength of 340 nm to produce a signal indicative of said concentration, the citrate content of the paper subsequently being calculated from said signal.

5. A method of determining on a discrete analyzer the citrate content of cigarette paper containing citrate ions substantially as hereinbefore described with reference to the example.

Printed for Her Majesty's Stationery Office by Croydon Printing Company (UK) Ltd, 6/87, D8991685.
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